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Credit author statement

Andrade, Victoria Soledad: main investigation, and writing of the initial draft

Wiegand, Claudia: investigation, supervision, methodology, reviewing and editing of the manuscript

Pannard, Alexandrine: investigation, methodology, formal analysis, visualisation

Gagneten, Ana María: funding acquisition, project administration, supervision,

Pédrot, Mathieu: investigation, methodology,

Bouhnik-Le Coz Martine: investigation, methodology,

Piscart, Christophe: conceptualization, funding acquisition, project administration, supervision, investigation, methodology, providing of resources for the study, co-writing of the initial manuscript.

Johnalprendi

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- Andrade, Victoria Soledad*^{1, 2,3}; Wiegand, Claudia³; Pannard, Alexandrine³; Gagneten,
 Ana María¹; Pédrot, Mathieu⁴; Bouhnik-Le Coz Martine⁴; Piscart, Christophe³
- 7
- 8 ¹Present address: Lab. de Ecotoxicología. Facultad de Humanidades y Ciencias.

9 Universidad Nacional del Litoral. Ciudad Universitaria, Ruta Nacional Nº 168, Km 472.4,

- 10 3000. Santa Fe, Argentina.
- ² Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Godoy
 Cruz 2290, C1425FQB, Buenos Aires, Argentina.

³ Univ Rennes, CNRS, ECOBIO - UMR 6553, 263 Avenue Général Leclerc, Campus de

- 14 Beaulieu, Bâtiment 14A, F-35000, Rennes, France.
- ⁴ Univ Rennes, CNRS, Géosciences Rennes UMR 6118, 263 Avenue Général Leclerc,
- 16 Campus de Beaulieu, Bâtiment 15, F-35000, Rennes, France.
- 17 *<u>victoria.s.andrade@gmail.com</u>.
- 18 +54-0342-155139910. Sarmiento 2873 2B, 3000, Santa Fe, Argentina.
- 19
- 20

21 Abstract

This study aimed to assess how bioturbation by freshwater benthic macroinvertebrates with 22 different biological traits alone or in combination could modify trace elements (TE) fate 23 24 between sediment and water, and if water TE concentration and animal TE content impair their body stores. Three macroinvertebrate species were exposed to TE contaminated 25 sediment for 7 days: the omnivorous Echinogammarus berilloni (Amphipoda), the 26 sediment feeding Tubifex tubifex (Oligochaeta) and the filter feeding Pisidium sp. 27 (Bivalvia). Treatments were one without invertebrates (control), two with amphipods or 28 29 mussels alone, and the combinations amphipod-mussel, and amphipod-mussel-worms. Water TE concentration increased significantly in 2 or 3 species mesocosms, concerning 30 mainly Rare Earth Elements, Cr, U and Pb, known to be associated to the colloidal phase. 31 By contrast, water soluble TE were not affected by animals. For both, amphipods and 32 mussels, TE body content increased with the number of coexisting species. For amphipods, 33 this increase concerned both, soluble and colloid-associated TE, possibly due to intense 34 35 contact and feeding from sediment and predation on tubificids. TE bioaccumulation in mussel was less important and characterized by soluble TE, with water filtration as most 36 plausible uptake route. Protein, triglyceride and Whole Body Energy Budget increased in 37 amphipods with the number of coexisting species (probably by feeding on mussels' feces 38 39 and tubificids) whereas triglycerides declined in mussels (presumably filtration was disturbed by amphipods). This study highlights interspecific interactions as key drivers 40 41 explaining both: TE bioturbation, depending on their water solubility or colloidal association, and the exposure/contamination of species through another species activity. 42



45 **1 Introduction**

Benthic macroinvertebrates bioturbate the sediment-water interface and are considered as 46 ecosystem engineers because they modify this environment and influence the availability of 47 resources and contaminants (Berckenbusch and Rowden, 2003). As many anthropogenic 48 pollutants find their way into aquatic ecosystems and into the sediments as final sink 49 (Eggelton and Thomas, 2004), bioturbation can modify their fate and distribution, either 50 directly by mixing or resuspending subsurface and surface sediment, or indirectly by 51 52 altering biogeochemical conditions (stability, redox conditions, organic content) (Blankson 53 and Klerks, 2016).

Within the hydrographic network, trace elements (TE) are distributed as dissolved, colloid, 54 or particulate form (Stumm and Morgan, 1996). Mineral constituents of sediments, 55 particularly the clay fraction, exhibit large surface areas, thus huge sorption capacity 56 regarding TE and organic compounds (Morley and Gadd, 1995). Moreover, redox-related 57 biogeochemical processes facilitate the transition of the particle bound TE to the pore water 58 and then to the pelagic zone (Xie et al., 2018). Remobilization of TE from sediments 59 depends on many factors, including the physico-chemical characteristics of the TE itself, 60 the content of organic matter and clay in the sediment, the hydrological flow, and non-the 61 least the bioturbation by the benthic community (Blankson and Klerks, 2017; He et al., 62 63 2017, 2019; Xie et al., 2018).

Between TE, rare earth elements' (REE) characteristics make them sensitive tracers of water-rock interactions, groundwater mixing, past redox conditions and soil phases contributing to TE sources (e.g. Davranche et al., 2011; Laveuf et al., 2012; Pédrot et al., 2015). REE in aqueous solution have an organic speciation and complexation in the presence of natural organic molecules (e.g. Marsac et al., 2010; Pédrot et al., 2008),

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exhibing specific REE signatures (Davranche et al., 2005; Tang and Johannesson, 2010).
Organic complexation of REE implies the development of a REE pattern in solution with a
middle rare earth element (MREE) downward concavity, associated to an absence of Ce
anomaly. Consequently, REE fingerprinting can allow to trace organic colloid
solubilization (Pédrot et al., 2010) and can be used to distinguish a differential
solubilization of organic colloids according to the activity of benthic communities.

Benthic macroinvertebrates bioturbate by feeding, locomotion, and using the sediment for 75 their habitat (Pearson, 2001; Mermillod-Blondin and Rosenberg, 2006). Consequently, 76 77 several functional groups have been proposed: biodiffusors, who randomly mix the sediment (e.g. amphipods); upward and downward conveyors, who move the sediment 78 vertically through feeding activity; regenerators, burrowing organisms that relocate 79 sediment (e.g. bivalves); and gallery diffusors, burrowing organisms that generate extensive 80 tunnels (e.g. Tubifex tubifex) (François et al., 2002). Few bioturbation studies have focused 81 on interspecific interactions affecting exposure to contaminants. For instance, Lumbriculus 82 variegatus (Oligochaeta) bioturbation augmented zinc resuspension, but reduced its 83 bioavailability for Chironomus tepperi (Chironomidae) (Colombo et al., 2016). Remaili et 84 al. (2016) found an increase in metals by bioturbation with the number of coexisting 85 species and a higher tissue concentration of chromium in bivalve in the presence of 86 87 amphipods.

88 Benthic macroinvertebrates are good sentinels of pollution on freshwater ecosystems 89 because of their relatively small action radius sensitivity to contaminants (Kiffney and 90 Clements, 1994) and some with relatively long-life cycles (Rosenberg and Resh, 1993), 91 moreover diverse ecological functions, thus taking part in a multitude of biological 92 interactions (Gerino et al., 2003). Even though interspecific interactions are complex

93 (McAuliffe, 1984), it needs to be revealed how these diverse functional groups –
94 individually or grouped – impact on contaminants fate, bioaccumulation and physiological
95 effects.

To address these questions, three sediment-associated macroinvertebrates were selected: the 96 omnivorous biodiffusor Echinogammarus berilloni (Amphipoda), the tunnel generating and 97 sediment feeding Tubifex tubifex (Oligochaeta) and the burrowing and filter feeding 98 Pisidium sp. (Bivalvia). Echinogammarus berilloni inhabits streams and rivers with a 99 preference for running waters (Piscart et al., 2007, 2011). Pisidium sp. lives in a wide 100 101 variety of habitats and seems to be of low sensibility towards high organic matter and decreased oxygen saturation (Herrington, 1962; Zeybek et al., 2012). Tubifex tubifex 102 inhabits fine sediment in freshwater lakes or lotic river sections, it resuspends the upper 103 sediment layer by feeding (Milbrink, 1987) and is relatively resistant to pollution 104 (Karikhoff and Morris, 1985). 105

The aim of this study was to assess how bioturbation by freshwater benthic 106 macroinvertebrates with different biological traits (i.e. omnivorous, filter feeder and deposit 107 feeder) alone or in combination (single to three species) could modify TE distribution 108 between sediments and water, using small mesocosms as exposure scenario. If bioturbation 109 enhances bioavailability it might also cause physiological effects in the organisms exposed. 110 111 Consequently, the specific goals were i) to analyze how macroinvertebrates bioturbation and their interspecific interactions affect the water TE concentration and the TE content in 112 113 invertebrates; and ii) to analyze if water TE concentration and animal content impair body stores of macroinvertebrates. 114

115

116 2 Material and methods

117 2.1 Sediment collection

Sediment was collected using a grab sampler in the Sélune River (Normandy, France) at the confluence with the Yvrande River (48°34'24.0"N 1°11'04.8"W), a tributary known by its historical TE pollution caused by industries (DREAL-Normandie, 2012). The sediment, remaining wet in its water, was sieved (Ø 2mm), homogenized and stored at 4°C two days before the experiment.

123

124 2.2 Organisms collection

Echinogammarus berilloni (Amphipoda) and Pisidium sp. (Bivalvia) where collected in the 125 Petit Hermitage River (48°29'16.1"N 1°34'15.8"W) located in the Villecartier forest, 126 considered as a reference site (Piscart et al., 2009). Specimens where maintained for one 127 day in water of their origin at $16.0 \pm 1^{\circ}$ C with aeration. *Tubifex tubifex* (Oligochaeta) where 128 bought from a pet shop (Grebil Co., France) and maintained in the same conditions. 129 Biofilm was collected in the Selune River upstream of the confluence with the polluted 130 Yvrande River using artificial substrates (glass plates) exposed for one month at 1m depth. 131 Biofilm was then inoculated on glass gems (previously washed with HCl 1N and Milli-Q to 132 remove TE) by incubating them during one month in a climate-controlled incubator with 133 $30.0 \pm 1 \mu \text{mol m}^{-2}\text{s}^{-1}$ of light, 12:12 light:dark regime at $20.0 \pm 1 \text{ °C}$. 134

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136 *2.3 Experimental design*

Flat round glass bowls (Ø14 cm) were filled with 100 mL of sediment and 200 mL of water from the reference site where invertebrates were collected. The approximate depth for sediment was 0.7 cm, and for water, 1.7 cm. The contact water/sediment surface was 154

7

 cm^2 . The water/sediment proportion was chosen according to the number of organisms and 140 their body sizes, and to ensure oxygen supply as no air bubbling was used to not add 141 additional water current. The bowls were left to settle in a climate-controlled incubator 142 $(30\pm1 \mu \text{mol m}^{-2}\text{s}^{-1} \text{ light}, 12:12 \text{ light:dark regime, } 16\pm1 \text{ °C})$. After 24h, invertebrates and 5 143 biofilm gems were added. Five combinations of organisms were performed with 5 144 replicates each: one without invertebrates (control), two with 10 individuals of either 145 amphipods or mussels alone, one combination of amphipod-mussel (10 individuals of 146 each), and a combination amphipod (10 individuals) - mussel (10 individuals) - worms (20 147 148 individuals).

The exposure lasted seven days to avoid any starvation (Foucreau et al., 2013) or oxygen depletion (Lawrence and Mason, 2001). From each bowl, one individual was frozen at -25°C for body stores and another specimen was dried at 65°C for 72h for TE content; four animals were frozen at -80°C for later enzyme analysis. Unfortunate none of the *T. tubifex* could be recovered. Samples of water and sediment were collected for nutrient concentration and TE content analysis.

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156 *2.4 Analysis of nutrients in the water*

Ammonium, nitrate and phosphate concentrations were measured at the beginning and at the end of the experiment from filtered water (GF/F) by colorimetric methods (Aminot and Chaussepied, 1983) using a Gallery Discrete Autoanalyzer (Thermo Scientific). The pH was measured with a combined Mettler InLab® electrode. Dissolved oxygen was measured with an Odeon portable apparatus (Ponsel) equipped with an optod probe.

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163 2.5 Trace elements determination

The elemental compositions of the sediment samples were determined at the CNRS Service 164 d'Analyse des Roches et des Minéraux (SARM), Centre de Recherches Pétrographiques et 165 Géochimiques (CRPG-CNRS, Nancy, France). After sample preparation (described in 166 167 Carignan et al., 2001), the major element concentrations were determined by inductivelycoupled plasma optical emission spectrometry (ICP-OES, Thermo ICap 6500) and the TE 168 concentrations by ICP-mass spectrometry (Thermo Elemental - X7 ICP-MS). The 169 bioavailable TE fraction in sediment was analyzed by leaching the water, acid soluble and 170 exchangeable fraction of sediment, following the first step of modified BCR extraction 171 172 (Pédrot et al., 2009). The first step was applied three times to 1 g of dried sediment sample, in ultracleaned 50-ml polypropylene centrifugal tubes. Extractant and procedure used: 0.11 173 M CH₃COOH, 40 mL, room temperature, shake for 16 h, centrifuged 3000 g for 30 min. 174

All collected waters were filtered using 0.2-µm-pore-size filter capsules (polyether sulfone 175 membrane Sartorius Minisart). Major cation and TE concentrations were determined by 176 ICP-MS (Agilent 7700x), using rhenium and rhodium as internal standard. Calibration 177 178 curves and accuracy controls were performed in accordance with Yéghicheyan et al. (2013), using river water reference material for cation and trace element with a large 179 compositional range (the international geostandard SLRS5, National Research Council of 180 Canada). The water samples were solubilized in 0.37 M HNO₃ with appropriate dilution(s) 181 182 regarding the ICP-MS quantifications limits. At the beginning of the experiment, TE concentration were assessed in sediment (including the BCR), animals (supplementary data 183 184 S1 and S2) and water; and at the end of the experiment, in water and animals.

For animal tissues, samples were prepared in a clean room in acid-washed digestion vessels (Savillex), respectively 50-mL tubes (24h in 1.5M HNO₃ at 45°C, 24h in deionized water at 45° C - repeated twice for the digestion vessels). Dry tissues were digested 5 times in subboiled nitric acid (14.6 M HNO₃), with or without hydrogen peroxide (H_2O_2), followed by 8h evaporation (95°C) each time. Final solids were re-solubilized in 0.37 M HNO₃ with appropriate dilution(s) regarding the ICP-MS quantifications limits. Typical uncertainties including all error sources were below 5% for all TE, whereas for major cations, the

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194 *2.6 Body stores determination*

uncertainty lied between 2% and 5%.

The concentrations in proteins, glycogen and triglycerides were measured via 195 196 colorimetric assays, as described by Foray et al. (2012). Each individual was lyophilized and weighed (Balance XP2U Mettler Toledo, Columbus, OH, d=0.1 µg). A volume of 600 197 µL of phosphate buffer was added to each sample, then homogenized for 1 min 30 sec at 198 25Hz (bead-beating device, Retsch[™] MM301, Retsch GbmH, Haan, Germany), and 199 centrifuged (500 g, for 5 min, 4 °C). 2.5 µL of the supernatant were collected for 200 quantifying protein content according to Bradford (1976) using bovine serum albumin as 201 202 standard.

The rest of the supernatant (597.5 μ L) was mixed with 900 μ L of a methanol-chloroform solution (2/1, volume/volume). After separation of the phases by centrifugation at 4 °C at 180*g* for 15 min, 300 μ L of chloroform was transferred into new microtubes for triglyceride assays, and the pellets were kept for Glycogen content. Chloroform was evaporated using a speedvac (MiVac DUO, Genevac, France) before redissolving the remains in 400 μ L of Triton-BSA buffer. The manufacturer's instructions were followed for the triglycerides colorimetric assay (Triglycerides, kit reference CC02200, LTA srl, Italy).

The glycogen content was assayed from the pellets by adding $1200 \ \mu$ L of fresh anthrone solution (1.42 g L-1 anthrone in 70% sulfuric acid). Samples were heated at 90 °C for 15 min, and absorbance was measured at 625 nm after a 2-or 4-fold dilution. Glucose was usedas standard.

The Whole Body Energy Budget (WBEB, $kJ.g^{-1}$) was calculated as sum of combustion of (17 $kJ.g^{-1}$ glycogen + 39.5 $kJ.g^{-1}$ triglycerides + 24 $kJ.g^{-1}$ proteins) expressed per gram of dry weight of the sample (Smolders et al., 2003).

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218 2.7 Statistical analysis

PCA was performed on the TE concentrations in water and the body content of invertebrates; dominant and physiologically relevant TE (Ca, K, Na, and Mg) were removed for the second PCA because they could be modified by physiological activities.

A PCA was also performed on nutrients concentrations, after scaling and reducing the data. 222 To highlight similar controlling processes (covariation, not causal-effect link) on nutrients 223 224 and TE to treatments, the co-variations of nutrients and TE ordinations from the first two axes of PCAs were analyzed through a co-inertia (Doledec and Chessel, 1994; Dray et al., 225 2003). The expected link here is not a causal – effect one, but covariations, which would 226 indicate similar controlling processes. The costructure between nutrients and TE is shown 227 on the plot of samples scores, on which the corresponding sample scores from both PCA 228 ordinations are linked by an arrow. The strength of the costructure is thus given for each 229 230 sample by the length of the arrow, with smallest arrows indicating maximum strength. To test statistically the global strength of the costructure, a permutation test of Monte Carlo has 231 232 been performed by randomly permuting rows and columns of one PCA and calculating the new co-structure (1000 permutations). All the analyses were performed using ade4 and 233 FactoMineR libraries implemented in R freeware (R Core Team, 2013). 234

11

The differences in nutrient or TE concentrations in water between treatments were assessed using one-way ANOVAs. The differences in TE body content and body stores were tested using two-ways ANOVAs with invertebrate species and species combinations as fixed factors. For all ANOVAs, Tukey's HSD tests were used to carry out post-hoc pairwise comparisons. All ANOVAs were performed using procedures from Statistica 7.1 (StatSoft, 2004).

241

242 **3 Results**

In all mesocosms and treatments, the physico-chemical parameters remained suitable for the animals. The oxygen concentration remained above 7.0 mg.L⁻¹ and pH ranged from 7.0 to 7.3 except for one mesocosm that increased until 7.7. For both, amphipods and mussels, survival rates were >90% in each mesocosm, except for one with only 80% of survival.

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248 3.1 Nutrient and TE concentrations in Water

Nitrate concentration increased significantly in the 2-species exposure (p < 0.008, Figure 1). Ammonium and phosphate concentrations both increased significantly in the 3-species exposure (p < 0.0029). Moreover, phosphate concentrations were significantly higher in all exposures containing amphipods (p < 0.003); a similar tendency was also observed for ammonium.

The first component (PC1, Figure 2a) of the PCA of the TE concentrations in the water represent 54.6% of the total inertia and is explained by most of TE, whereas the second component (PC2) explains 16.3% variations of a group of 9 TE (B, Ba, Cd, Co, Li, Mn, Mg, Sr, and Ca). Eigenvalues are shown in Figure 2b. The water in mesocosms without invertebrates (controls) and the mesocosms with only one invertebrate species (mussels or

amphipods) had similar TE concentration, even though mesocosms with amphipod tended 259 to have a higher content in TE associated with the second component (Figure 2c). The 260 water of mesocosms with 2 or 3 invertebrate species had a significantly higher TE 261 262 concentration than mesocosms with only one species (P-values < 0.05); for 76.7% of metals, these TE being associated with the first component. This group include REE, Fe, 263 Cr, U, and Pb, which increased significantly with number of species (Figure 3a). Moreover, 264 a MREE downward concavity was evidenced in all modalities, with an absence of 265 evolution of Ce anomaly (data not shown). The increase of TE concentration is particularly 266 267 strong for TE associated to organic colloids (e.g. Cr, Pb, REE and U), which are all on the right part of the PCA, whereas true dissolved TE (Li, B, Ba, Ca, K, Mg, Mn, Na and Sr) 268 were not affected (Figures 2a and 3d,e). A co-inertia (Figure 4) has been performed from 269 the previous PCA on TE concentrations and from a PCA on nutrients concentrations. Both 270 ordinations showed a high percentage of variance on the first two axes, with 90 % for 271 nutrients and 70 % for TE. The first axis of the co-inertia separates the three species 272 273 treatments on the left part of the co-inertia from controls and mussels on the right part, with Amphipods and two species in the middle (Figure 4). Compared to the initial PCA, some 274 TE are highlighted by the co-inertia, such as Rb for the first axis and Cu, Zn and As for the 275 second axis (Figure 4). The dispersion of the samples on the plot moreover increased with 276 277 the number of species.

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279 *3.2 TE content in animal's body*

The mean TE content in non-exposed animals were relatively low. The ratios between the TE content in mesocosms with one species VS non-exposed animals ranged from 1 to 10 for amphipods and from 0.4 to 2 for mussels.

The TE body contents for exposed animals are ranged between the two first components 283 (PC1 and PC2) representing a total of 73.6% of the inertia (Figure 5a). The first component 284 (PC1) represent 51% of the total inertia and is explained by most of TE, whereas the second 285 286 component (PC2) explains 12.6% variations of a group of 13 TE (As, B, Ba, Cd, Co, Cu, Eu, Fe, Mn, Rb, Sb, Sr, and Zn). Eigenvalues are shown in Figure 5b. Apart from Ca, 287 which is higher in mussels ($F_{1,2,24} = 462.8$; P < 0.001), the overall body content of TE was 288 significantly higher in amphipods compared to mussels (Figure 5c). Concerning single TE, 289 apart from Ni (P = 0.129), and Cd (P = 0.454), all others were significantly more 290 291 concentrated by amphipods than by mussels (P-values < 0.003). The body content in animals increased according to both, the species considered and the number of species in 292 mesocosms. For amphipods, increase in TE concerned mainly TE associated with the 293 second axis (e.g. Rb, Cu, Sr, and Mn), whereas for mussels this increase was mainly 294 295 associated with the TE depicted on the first component (e.g. Al, Ce, Nd, and La) (Figure 296 5c).

Amphipods' TE body contents were not correlated with TE concentration in water.
However, for mussels, significant correlations, most of them being negative were found
with 22 TE, including all REE, V Be, Cr, Fe, Ga, Pb, U, Y, and Zn.

300

301 *3.3 Body stores*

Protein content differed significantly between the species but not according to the treatments, despite a slight increase in amphipods with number of coexposed species (Figure 6a). Glycogen content did not differ significantly, neither between species nor between combinations of species (Figure 6b, P-values > 0.165). Triglyceride contents did not differ significantly between amphipods and mussels in mesocosms with one or two species (P-values > 0.276) but became significant in mesocosms with three species (Figure
6c, c; P-values <0.018).

The WBEB was not significantly higher in amphipod than in mussels in mesocosms with one species (Figure 6d) but was significantly higher in the two and three species exposures (P = 0.004). TE concentrations in water did not significantly influence the WBEB, whereas significant negative correlations between WBEB and TE body content were observed. For amphipods, WBEB was negatively correlated with 22 TE (all REE, Fe, Ga, Pb, Sc, Si, U, V, and Y). For mussels, negative correlations were only found with Li (P = 0.027), Sb (P = 0.036) and Sc (P = 0.043).

316

317 4 Discussion

318 *4.1 Nutrients and TE in Water*

Bioturbation of the upper sediment layer by amphipods and oligochaetes increased the 319 concentration of ammonium and phosphate in the water, confirming studies by Mermillod-320 321 Blondin et al. (2008). Moreover, excretion by both organisms might have contributed, especially because animal biomass increased with the number of species in mesocosms 322 (Fukuhara and Yasuda, 1989; Gardner et al., 1981; Nalepa et al., 1983). Amphipods are 323 moreover suspected to have benefited from T. tubifex as nutrient source, thus their 324 325 excretion and the decay of the oligochaetes contributed to the high values in these exposures. Contrarily, mussels alone did not change any of their energy storage, probably 326 327 due to their lower activity in bioturbation.

Bioturbation similarly modified the TE content in the water, again in particular in exposures including amphipods. Li, B, Ba, Ca, K, Mg, Mn, Na and Sr, were not affected by the animals but crossed the benthic-pelagic border by diffusion. Indeed, these elements are

known to be not or slightly associated to the colloidal phase, resulting in a distribution 331 within the truly dissolved phase (< 1 nm) (Pédrot et al., 2008). Within the dissolved 332 fraction in river waters ($< 0.22 \mu m$), Fe-organic colloids are major carriers of TE with 333 334 lower solubility (e.g. Cr, U, Pb and REE) (Pokrovsky and Schott, 2002; Sholkovitz et al., 1995). REE fingerprinting confirmed their organic complexation, and the increase of REE 335 concentrations with the number of species showed an increase of organic colloid 336 solubilization. Cr, U and Pb and REE were the most affected by the presence of organisms, 337 indicating that bioturbation was the main factor releasing TE associated to colloids (less 338 339 bioavailable) from the sediments. This study confirms that bioturbation contributes to TE redistribution from the sediment into the pelagic phase thus increasing its bioavailability. It 340 moreover contributes to the understanding of the specificity of this process depending on 341 the water solubility or colloidal association of the TE. 342

The contribution of each bioturbator type however, varied considerably. The least 343 contributed the mussels, which even though living buried in the sediment seemed to have 344 used the overlaying water for their respiration and filtering activities without causing 345 disturbances of the sediment. The amphipods conversely, foraging by moving their 346 sideways compressed body with their legs, apparently swirled sediment particles. 347 Tubificids might have added to this bioturbation by their movements and moreover by 348 349 feeding from deeper sediment layers and rejecting the feces into the overlaying space (He et al., 2019; Karlckhoff and Morris, 1985), but we could not prove it with our experimental 350 351 set up. Similarly, comparing a range of benthic species, Gammarus pulex bioturbation activity was found to be higher than that of T. tubifex, which in turn liberated higher 352 quantities of Cu from the sediment and considerably increased the toxicity (van der Meer et 353 al., 2017). 354

355

356 4.2 TE content in animal's body

TE bioavailability to aquatic invertebrates is complex as both uptake routes (waterborne 357 358 and dietary) exist in parallel (Brix et al., 2011). The dominant pathway may vary depending on species, permeable surfaces, feeding behaviour, metal, concentrations and available 359 binding sites (De Jonge et al., 2010; Marsden and Rainbow 2004). Generally, amphipods 360 seemed to accumulate more TE than mussels, confirming that they are physiologically good 361 bioaccumulators (Geffard et al., 2010; Schaller et al., 2011). Amphipods foraging 362 363 behaviour in intense contact to the sediment exposes their thin articular membranes and gills as uptake routes for TE. Secondly, feeding activities, especially predation on 364 invertebrates such as oligochaetes (Piscart et al., 2011), tubificids in this scenario, also 365 contamination with TE through biomagnification. 366 promotes their Contrarily, bioaccumulation in mussels was lower and characterized by soluble TE (e.g. As and Zn), 367 whose main uptake route was presumably by water filtration. 368

For both, amphipods and mussels, the whole TE content increased with the number of 369 species in the mesocosms. TE affected by the experimental conditions were however not 370 the same. In mesocosms with amphipods and mussels, the water soluble TE increased in 371 amphipods which could be explained by the filtering activity of mussels, clearing the TE 372 373 from the water column but concentrated them with their faeces near the sediment (Vaughn and Hakenkamp, 2001) where they are consumed by amphipods (Gergs and Rothaupt, 374 375 2008). In those mesocosms, for mussels the soluble TE content decreased whereas colloidal TE content increased, according to its increase in the water caused by amphipods' 376 bioturbation. Finally for both, amphipods and mussels, the increase in TE content were 377 more important in the presence of tubificids. The tubificids' bioturbation activity might 378

have transferred TE from deeper layers of the sediment to the pelagic zone (He et al., 2019; 379 Mermillod-Blondin et al., 2007; Reible et al., 1996), or modified physical properties of 380 sediment, e.g. redox potential (Graf and Rosenberg, 1997), increasing their bioavailability. 381 382 Moreover, for the amphipods increase could have been via biomagnification (Mermillod-Blondin et al., 2004). Our study confirms the increase of TE transport over the sediment 383 water interface by bioturbation (Remaili et al., 2016) and moreover demonstrates how 384 increasing interactions between invertebrates affect TE accumulation depending on species 385 and TE partitioning between colloidal and dissolved. 386

387 *4.3 Body stores*

In our exposure scenario with environmentally low TE concentrations, body stores of 388 neither amphipods nor mussels were strongly impacted. On the contrary, body stores tended 389 to increase with the number of species coexisting. This is consistent with previous studies 390 showing a low effect of heavy metals on triglycerides and protein in the freshwater 391 gastropod Potamopyrgus antipodarum exposed even at higher concentration of Cd (0.8 392 $\mu g.L^{-1}$) and Zn (50 $\mu g.L^{-1}$) (Gust et al., 2011) than in our experiment (highest mean 393 concentrations measured: Cd = 0.24 and $Zn = 35.2 \ \mu g.L^{-1}$). Moreover, amphipods and 394 mussels are known to be good metal bioaccumulators able to sequester metals by cellular 395 ligands such as metallothioneins, lysosomes, and mineralized organically based concretions 396 397 without being considerably harmed (Geffard et al., 2010; Langston et al., 1998).

The correlation between protein and triglyceride contents with number of species exposed lead to a significant increase of WEBB in amphipods whereas it declined in mussels. This pattern highlights better experimental conditions for amphipods as they benefitted from the presence of tubificid preys (Mermillod-Blondin et al., 2004) and hence increased their energy. Amphipods might also have benefitted from mussels which concentrate the food

items (e.g. bacteria) in the surface of the sediment (Vaughn and Hakenkamp, 2001) or 403 produce consumable feces (Gergs and Rothaupt, 2008). In turn, physical interactions 404 between amphipods and mussels might have disturbed the filtration rate of mussels and 405 406 lead to the observed decrease in their triglyceride content (and the low change in TE concentration). The pattern observed for glycogen in amphipods is more complex with a 407 strong decrease in mesocosms with amphipods and mussels together. This might be due to 408 the increase of certain TE in the water of these mesocosms, especially for the toxic ones 409 410 such as As, Cd, Cu, and Zn (Borgmann et al., 2005). Glycogen is used by amphipods to 411 cope with harsh environmental conditions and may strongly decrease in presence of toxic compounds (Dehedin et al., 2013; Maazouzi et al., 2011; Marmonier et al., 2013). The 412 physiological state of the organisms in terms of their energetic stores was not that strongly 413 impaired by the TE during the short exposure time (7 days) and the relatively low TE 414 concentrations in the sediment. Nevertheless, some of the TE were negatively correlated 415 with body stores of amphipods (Fe, Ga, almost all REE, Sc, Th, V, and Y) and mussels (B, 416 Sb. Sc and Th). However, further studies are needed to understand the underlying 417 mechanisms and the effects of TE over a longer term. 418

419

420 5 Conclusion

Our study confirmed the strong effect of bioturbation of sediments by aquatic invertebrates on the transfer of colloidal and organic matter associated TE to the pelagic compartment, whereas the soluble fraction of TE was not affected. We also highlighted that interspecific interactions appear to be a key driver explaining both the resuspension of TE, depending on their characteristic, and the exposure/contamination of species through another species activity. Previous studies combining several species with different trophic positions focused 427 on the biomagnification of TE along the food web, but our study revealed that a 428 combination of several species would also control indirect contamination via increased 429 bioavailability of TE by bioturbation activities. Moreover, we could not demonstrate high 430 energy allocation in organisms exposed to TE but amphipods however, clearly benefitted 431 from the additional energetic resources provided by tubificids. Further studies are required 432 to measure the intensity of these interactions with more and or other functional feeding 433 groups.

434

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673 Figure legends

Figure 1. Mean $(\pm$ SD) concentrations of nitrate (NO₃), phosphate (PO₄) and ammonium (NH₄) in water according to the five experimental conditions. Significant differences are indicated by letters and numbers.

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Figure 2. PCA on trace elements in water. (a) Correlation circle. (b) Eigenvalues. (c)
Distribution of experimental conditions on the PC1–PC2 axes. Mean position of each
experimental condition (white circles) are positioned at the weighted average of the five
replicates (black dots).

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Figure 3. Mean (± SE) of selected TE concentrations in water according to the five experimental conditions: (a) total content in Rare Earth Elements (REE), (b) iron concentration, (c) Sum of Cr, U and Pb, (d) total content in highly Soluble Trace Element (STE) including (Li, B, Ba, Ca, K, Mg, Mn, and Sr), and (e) Sodium on its own.

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Figure 4. Projection of two first axes of co-inertia analysis (COIA) after a double PCA: (a) nutrients concentrations, (b) TE in water (REE are shown in the box), and (c) samples scores by modalities and the arrows showing the change from one view to the other, indicating the strength of the links.

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Figure 5. PCA on trace elements in animals: (a) Correlation circle, (b) Eigenvalues, (c)
Distribution of experimental conditions on the PC1–PC2 axes. Mean position of each

experimental condition (white circles) are positioned at the weighted average of the five 695 696 replicates (black dots).

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698 Figure 6. Mean (\pm SE) content in (a) proteins, (b) triglycerides, (c) glycogen, (d) mean (\pm

SD) values for Whole Body Energy Budget (WBEB) according to experimental conditions 699

700 for amphipods (dark bars) and mussels (white bars). Significant differences are indicated by

letters. 701

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Figure 3.



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Figure 5.

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Highlights 1

- 2 - Macroinvertebrates bioturbation affects trace elements fate among sediment and water
- 3 - Trace elements bioturbation depends on their solubility or colloidal association
- 4 - Trace elements bioturbation depends on macroinvertebrates biological traits
- Interspecific interactions are key drivers for trace elements bioaccumulation 5
- Bioturbators increase trace elements bioavailability for coexisting specie 6

Declaration of interests

 \boxtimes The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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